

BRANCH INITIATION IN THE LICHEN *CLADONIA*
CRISTATELLA (BRITISH SOLDIERS)

SAMUEL HAMMER

College of General Studies, Boston University,
871 Commonwealth Avenue, Boston, MA 02215

ABSTRACT. Branch initiation in the British Soldier Lichen (*Cladonia cristatella*) follows a sequence of discrete developmental stages. Branches arise from dichotomous divisions of a meristem-like apical bundle of fungal cells. Early ontogenetic phases are predictable and controlled by the activity of the fungal meristem. One source of variability in mature thalli is heterochrony; the branching process may be synchronized differently in different thalli. In some thalli the meristem may divide early in ontogeny, in which case the branches are relatively long and distinct. In other thalli the meristem enlarges but does not divide until later in ontogeny. In this case branching is delayed and the branches may be shorter and less distinct. Both patterns of timing for branch initiation produce mature thalli with a morphology that is unique to *C. cristatella*. The early ontogeny in *C. cristatella* is similar to that of taxa that are presumed to be only distantly related. This suggests that branching may be canalized in *Cladonia* species, and that certain branching patterns represent primitive characters in the Cladoniaceae. Morphological variability in *C. cristatella*, which is based partly on heterochrony, has both taxonomic and evolutionary implications.

Key Words: branching patterns, Cladoniaceae, growth dynamics, lichen evolution

Cladonia cristatella Tuck. is a common endemic lichen species of New England that is characterized by great morphological variability. Thomson (1968) listed 12 forms for this species, most of which were based on morphological differences. While these morphological *formae* are not taxonomically significant, they indicate the range of variation perceived in this species by earlier workers. Some of the variation is based on branching patterns, the interpretation of which has caused taxonomic difficulties in this and other Cladoniaceae species (Hammer 1995). Yet branching in *C. cristatella*, though variable, is predictable in its early stages. In *C. cristatella*, as in other Cladoniaceae species, thallus form is produced by definite developmental patterns that produce simple cuplike structures and branches. The simplicity of thallus form in the Cladoniaceae led systematists in the past half century to consider morphology to be of little utility taxonomically. It has

been generally but mistakenly perceived that morphological characters of the species are not informative, and that a solution to this problem might be found in chemotaxonomy. Hale (1984) stated that genera in families with a fruticose habit (such as the Cladoniaceae) had few ontogenetic characters. This statement may have been prompted because of the difficulty in tracing morphogenesis in complex lichens: the relationship of morphology in mature *Cladonia* thalli to early development is often unclear. As a result there has been little work on the comparative ontogeny, anatomy, and architecture of the Cladoniaceae. Subsequently, the evolutionary relationships of *C. cristatella* and other Cladoniaceae are unknown because comparative morphological data are scarce. Early workers such as Robbins and Sandstede (see Evans 1930, 1935) noted and named minor morphological differences in populations of *C. cristatella*, while ignoring the underlying developmental similarities of the various forms they described. Similar to other Cladoniaceae species, the taxonomy of *C. cristatella* was thereby burdened with a complicated nomenclature that did not adequately reflect its biology. But the evolutionary biology of *C. cristatella* is reflected in its development. The goal of this paper is to elucidate the developmental biology of *C. cristatella* through a description of branch initiation.

MATERIALS AND METHODS

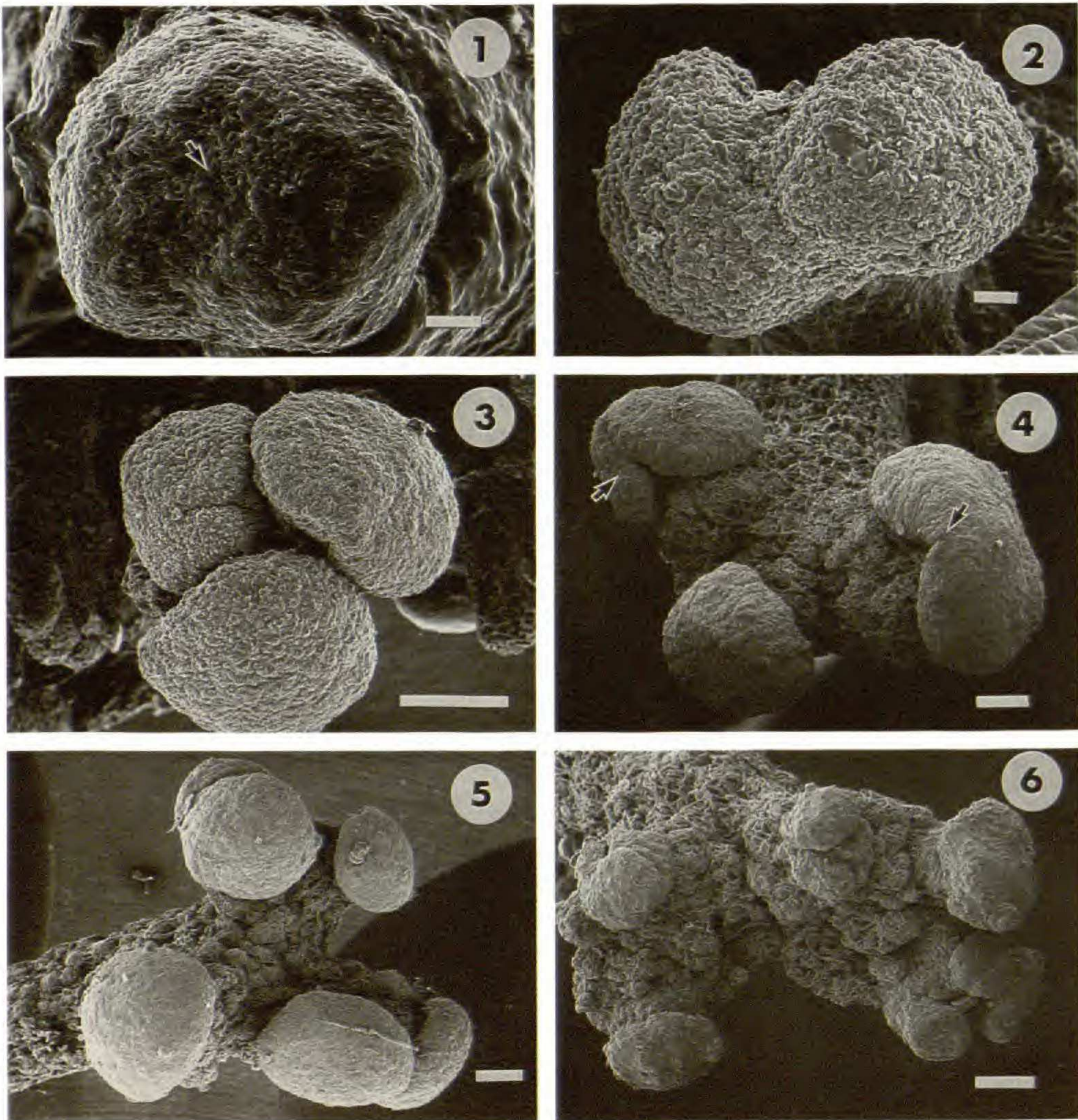
Several hundred thalli of *Cladonia cristatella* were examined for this study. Particular attention was given to the podetium which is the erect "secondary" thallus of *Cladonia* species. Immature, unbranched podetia, most of which were <1 cm tall, were studied. Mature podetia were examined as well. Most of the podetia and all of the thalli that were dissected came from recently collected specimens. These specimens were collected from a variety of substrates in pine-scrub oak forests in Plymouth and Barnstable Counties, Massachusetts. Many preserved specimens at the Farlow Herbarium (FH) also were examined and annotated. These include (but are not exclusive to) *Ahmadjian 2366*, *Culberson 6768*, *Evans 1858*, *Hammer 6015*, *Oakes s.n.* (lectotype), *Riddle 208*, *Robbins s.n.*, and *Sheldon s.n.* Notes were taken, and many of the specimens were sketched under a dissecting microscope. Scanning electron micrographs (SEM) were prepared from

approximately 30 freshly collected specimens. See Hammer (1996a) for details on SEM methodology.

The terminology for descriptive studies in lichen morphogenesis is not yet an established convention. This is a problem because lichen form, particularly in Cladoniaceae species, may be plant-like. The shrubby thallus (body) of many of these lichens tempts comparisons with vascular plants, but lichen architecture is based on different units from plants (Hammer 1997). Developmental morphology of lichens is an evolving discipline, and here I follow certain terminological conventions used in previous papers. For example, the cluster of apical cells that comprises *meristem-like tissue* will be called the “*meristem*” or “*meristem bundle*.” “*Bundle*” is a general term for a group of similarly oriented cells that extends parallel with the long axis of the branch (Hammer 1993a, 1996b, 1997; Letrouit-Galinou 1966, 1968). In studies of vascular plants the term “*meristem initial*” sometimes refers to a single apical cell. In this paper the term refers to *the developmental unit* of up to five branch initials, as in Hammer (1996c, 1997). The term “*division*” refers to the splitting of the meristem tissue, and is not associated here with *cell* division. In Hammer (1996a) “*division*” was used interchangeably with “*split*.” “*Division*” is used here as a noun (e.g., the division of the meristem tissue) and “*split*” is used to describe an action, such as when the meristem splits into two parts.

RESULTS

Branch initiation in “early-branching” podetia of *Cladonia cristatella* is determined by the ontogenetic sequence of the apical fungal meristem. The fungal meristem is an aggregation of fungal cells ca. 50 μm in diameter. Before division, the aggregation enlarges to ca. 100 μm and a division furrow forms at the approximate center of the meristem bundle (Figure 1). The bundle later splits along this furrow. The first division produces two more or less equal bundles (Figure 2). The orientation of the bundles changes during development so that the longitudinal axes of the bundles are approximately perpendicular. The next division furrow is oriented approximately perpendicular to the initial division furrow. A division along this furrow, which is the second division to occur in the sequence, produces a meristem initial of three bundles (Figure 3). These



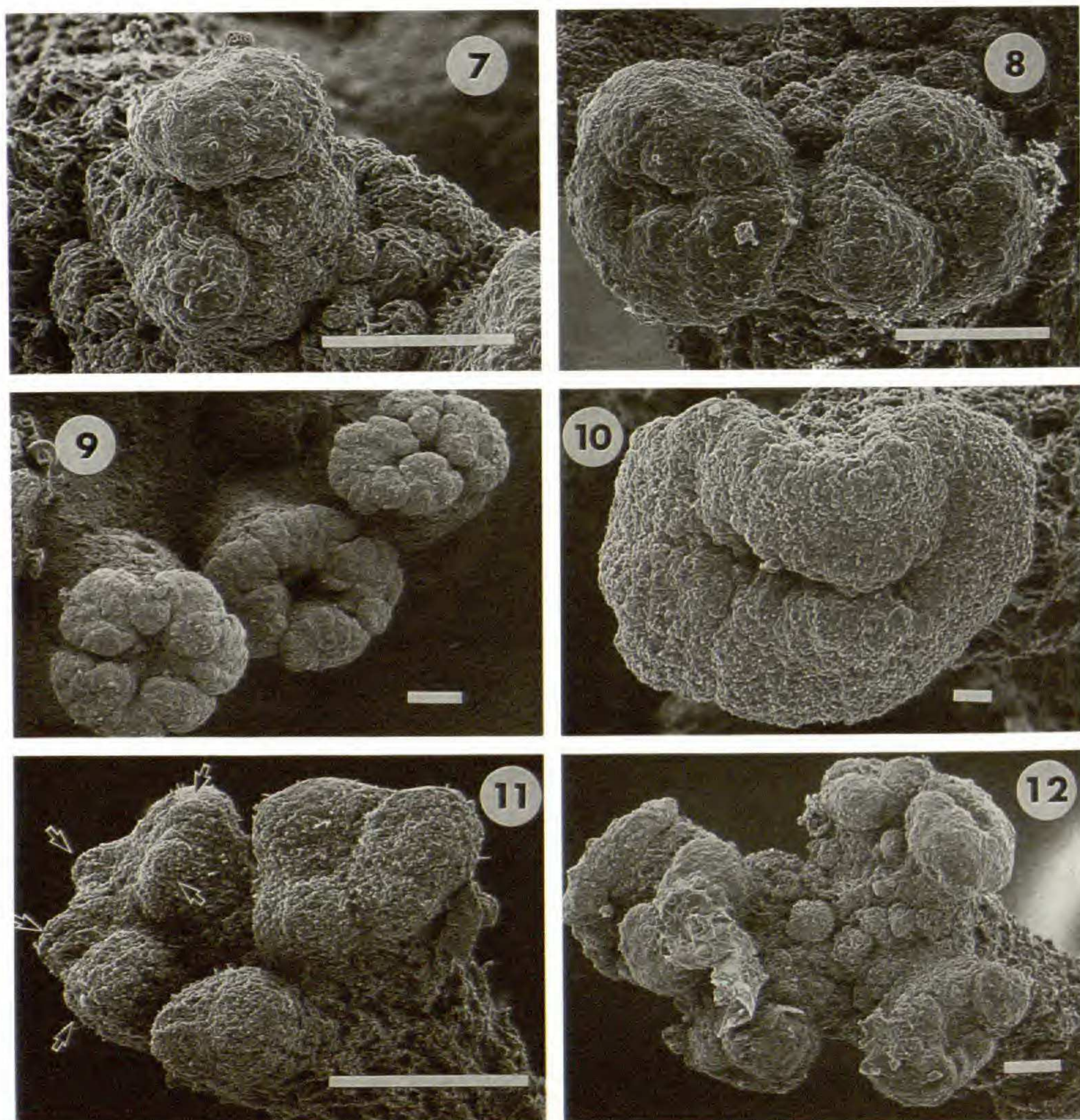
Figures 1–6. Micrographs of fungal meristem in early branching *Cladonia cristatella* (SEM). 1. Apical fungal meristem in earliest stage, before division. Note longitudinal furrow (arrow). 2. Meristem later in ontogeny. First division has occurred but meristem has not split. 3. Three meristem bundles produced by two divisions. 4. Branch initiation by separation of meristem bundles and elongation of thallus beneath them. Note developing division furrows on two bundles (arrows). 5. Further divisions of meristem after elongation. 6. Complete meristem initial with meristem regions that will continue to divide. Scale bars in Figures 1 and 2 = 10 μm . Other scale bars = 100 μm .

bundles may give rise to branches, or they may enlarge and undergo further divisions. Branch initiation occurs when the meristem bundles separate and the lichenized tissue beneath them elongates (Figure 4). Thus, the process that is laid down by the fungal partner is continued in the symbiotic thallus. During any intermediate step (for example, Figure 5), meristem

divisions may slow or stop, and branches may be produced. The branches that arise from intermediate steps usually are produced at approximately the same rate. Thus, two to five branches on the same podetium usually are at the same approximate stage of development. The meristem initial is complete when there are five more or less distinct apical meristem bundles (Figure 6). After this point, any meristem bundle of the initial may continue to split, giving rise to branches. Alternatively, it may enlarge without splitting. Finally, any bundle from the meristem initial may remain quiescent. Subsequent divisions may occur at any time and usually are not synchronous. The result is a podetium with five or more branches. In mature podetia with more than five branches, the branches thus may be observed in various stages of development.

In “late-branching” podetia, described below, the meristem tends not to divide as rapidly as in “early-branching” podetia. The initial stage of meristem development may resemble “early-branching” podetia (Figure 7). When the meristem splits, the resulting filial bundles (Figure 8) may continue to grow rather than dividing. They are usually larger than 100 μm in diameter and they may grow to nearly 500 μm . Alternatively, one of the two bundles may split, giving rise to branches while its filial bundle continues to grow (Figure 8). Series of indistinctly divided bundles may be produced in a roughly linear series (Figure 9). These may appear as short protrusions along the margin of a cuplike structure. They also may produce cuplike structures themselves. Any one of these, or part of one, also may split and form branches. The result is that the mature thallus appears to have an irregular branching pattern.

The large bundles appear to have several actively growing areas. There may be some distinct divisions that resemble the furrows described above but the “late-branching” meristem develops as a contiguous, roughly circular, convoluted structure (Figure 10). Branching in mature “late-branching” podetia is indistinct. The characteristic five-bundle meristem initial may or may not be formed in “late-branching” podetia. Where characteristic divisions of the meristem bundle do occur, the five-bundle meristem initial may be the result. The large meristem bundles produce lateral growth (Figures 11, 12) but relatively little vertical growth. Smaller bundles (<100 μm diam.) permit vertical growth.



Figures 7–12. Micrographs of fungal meristem in late branching *Cladonia cristatella* (SEM). 7. Early meristem bundle. Division furrows have formed but meristem has not split. 8. Two filial meristem bundles. Bundle on right has begun to split, initiating branch growth. 9. Series of indistinctly divided bundles in a roughly linear series. 10. A roughly circular, convoluted meristem structure. 11. Indistinct five-unit meristem initial has begun to form in the left-hand structure (arrows). 12. Indistinct meristem initial with short branches. Note four meristemetic regions that have not split, or which have partially split. Scale bar in Figure 10 = 10 μm . Other scale bars = 100 μm .

DISCUSSION

Mature thalli of *Cladonia cristatella* have a variable though broadly characteristic morphology. For example, most mature podetia of this species are branched. Branching patterns vary, and podetia may be unbranched, sparingly branched, or abundantly branched. Branching may occur around the margins of a cuplike

structure or it may occur independently, with branches arising at the apex of the podetium. Growth seems to be constrained by the size of the continuous meristem bundles. Large meristem bundles either divide or produce cuplike structures but they do not produce branches. By contrast, vertical growth is prevalent where meristem divisions are more frequent and occur earlier in ontogeny, which also was observed in verticillate *Cladonia* podetia (Hammer 1996b). However, the overall variability in *C. cristatella* is constrained by patterns of fungal meristem ontogeny. Branching and vertical growth both are determined by the meristem. The meristem bundle may enlarge and split dichotomously several times in relatively rapid succession, or it may grow rather slowly before splitting. In addition to the variability in mature podetia, early ontogeny in *C. cristatella* is variable. It is unusual to detect this much variation early in ontogeny, as most species in the Cladoniaceae follow a more highly constrained developmental plan. Yet the source of variability in *C. cristatella* is based on different expressions of synchronization. Jahns (1988) may have recognized this when he described the "faulty coordination" of development in *C. cristatella* that was growing in culture.

The varied timing of branch initiation in *Cladonia cristatella*, which occurs early in ontogeny, suggests a source of variation that may be independent of environmental influences. Variability in early ontogeny among thalli growing very close together suggests a heterogenous gene pool within populations of *C. cristatella*. Ahmadjian (1993) found that clones of one thallus were genetically heterogenous. It can be assumed that many genotypes comprise the species complex that is broadly identified as *C. cristatella*. Phenotypic variability among mature thalli is, therefore, a reasonable expectation. Yet certain ontogenetic features unite all of the phenotypes. Their expression at maturity simply depends upon the synchronization of developmental steps. Synchronization of growth in other *Cladonia* species may provide the basis for taxonomic differences (Hammer 1997). If variations in the development of *C. cristatella* are the result of heterochrony, then heterochrony may play a role in the evolution of this species.

It is noteworthy that the early ontogeny of *Cladonia cristatella* is similar to species that are presumed to be only distantly related. For example, the development of the early-branching variant is similar to species in *Cladonia* section *Perviae* (Del.) Matt. (Hammer 1996a). The five-bundle meristem initial also was described in the

genus *Cladina* Nyl., for example, in *C. subtenuis* (Abb.) Hale & Culb. (Hammer 1997). While the mature form of these species is different from *C. cristatella*, their pattern of branch initiation is similar. In species in section *Perviae* and in *Cladina subtenuis*, the development of a meristem initial of five fungal bundles through irregular dichotomous divisions is the same as in *C. cristatella*. Branching may continue indeterminately from the meristem initial in all of these species but it is least predictable in *C. cristatella*. In the other taxa, the meristem appears to "rest" after the five-bundle initial has formed. In *C. cristatella* the period of quiescence is short and the meristem initial is indistinct. New divisions form soon after the meristem initial has been established.

The "late-branching" variant of *Cladonia cristatella*, in which the meristem enlarges, becomes convoluted, and slowly splits, has a growth dynamic that is similar to *Cladonia grayi* Merr. While the mature thalli of these species are only vaguely similar (*C. cristatella* may produce cuplike structures while *C. grayi* invariably forms cups), early meristem development in both species is the same. *Cladonia grayi* develops from the same convoluted meristem as *C. cristatella*. These examples provide evidence in lichens to support von Baer's third law (Baer 1828), which states that early developmental stages in related taxa are more similar than later ones (see Wallace 1988).

Ontogenetic information from comparisons of *Cladonia cristatella* with other species has taxonomic and evolutionary implications. Classification is one concern. Generic and infrageneric (sectional) boundaries in the Cladoniaceae are based upon the morphological criterion of "open" vs. "closed" cups and axils (Hammer 1995; Mattick 1938, 1940). But the criterion may not be appropriate for this level of taxonomic distinction. Axillary openings may differ in closely related species (Hammer 1993b, 1996a), a result of differing growth rates. The occurrence of openings is not consistent. They may form in species classified as "closed" axil, and they may not appear in "open" axil species. Finally, this criterion is based on the appearance of mature podetia, which is variable and confusing.

Early ontogeny of branch initiation may provide a robust character upon which to base taxonomic groupings. Branch initiation is the same in *Cladonia cristatella* ("closed" axil) and in *Cladina subtenuis* (an "open" axil species). This leads to questions about the evolution of branching in the Cladoniaceae. Have similar

branching patterns arisen more than once? Alternatively, might branching patterns be canalized? The fungal meristem is homologous in *Cladonia* and *Cladina*, and development is the same in both genera. The similar development of the meristem initial in *Cladina subtenuis* and *Cladonia cristatella* suggests that these taxa might not represent two distinct genera.

From an evolutionary standpoint, it is helpful to consider the biogenetic law of Haeckel, which states that general characters are primitive (plesiomorphic) and less general characters are advanced (apomorphic; Nelson 1978). In the Cladoniaceae, the five-part meristem initial is more general (common) than axillary openings. Axillary openings arise from the development of branches, not the other way around, and branched species may not produce openings. May we infer that branched thalli are an evolutionary advance over non-branched thalli in the Cladoniaceae? Evolutionary advance may be inferred from specialization, or from the appearance of autapomorphies. Some lichens, such as *Cladina* species, develop in distinct, highly predictable patterns that Ahti (1961) called "regular." The mature thallus of these species is specialized for photosynthetic and reproductive functions (Hammer 1997). Development in *Cladonia cristatella* is less regular. The mature thallus also is less specialized. *Cladonia cristatella* possesses what can be considered to be a suite of primitive characters. The timing of its early ontogeny is variable. Its unspecialized program of meristem development includes growth patterns that form cuplike structures *and* branches. The early ontogeny of the meristem is similar to other, more specialized species. It seems plausible that species with a related but more specialized growth pattern represent an evolutionary radiation rooted in *C. cristatella* and its relatives. Branching, cup formation, and axillary openings are determined by the early ontogeny of the meristem. The exclusively fungal meristem may prove to be informative about the evolution of morphological characters in the Cladoniaceae.

ACKNOWLEDGMENTS. I thank D. H. Pfister for providing access to collections at the Farlow Herbarium and Reference Library. Grants from the Massachusetts Natural Heritage and Endangered Species Program (NHESP) provided partial funding for collecting and photographing the specimens. I acknowledge the patient skill with which Mr. Edward Seling assisted me in preparing SEM micrographs for this paper.

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